INTERFERON GAMMA INDUCING FACTOR BASED VACCINE AND USE OF SAME FOR PROTECTIVE IMMUNITY AGAINST MULTIPLE SCLEROSIS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to the treatment of multiple sclerosis and, more particularly, to interferon gamma inducing factor (IGIF) based vaccines for effecting same.

Based on their cytokine profile $CD4^+$ T-cells can be divided to Th1 cells that produce large amounts of interferon gamma (IFN-γ) and TNF-α, and, to a much lesser extent, IL-4 and IL-10; Th2 cells that produce IL-4, IL-10, and IL-13 and, to a much lesser extent, IFN-γ and TNF-α (1-10), and the newly defined Th3 cells that produce significant amounts of transforming growth factor beta (TGF-β) and have been associated with oral tolerance (11).

Th1 cells selected in response to various auto-antigens transfer T-cell mediated autoimmune diseases, whereas IL-4 secreting Th2 cells, selected in response to these same antigens, either inhibit or exert no profound effect on the inflammatory process (5, 12-24).

High levels of IFN-γ and low levels of IL-4 positively select for Th1 cells, whereas, low levels of IFN-γ together with high levels of IL-4 mediate Th2 selection (1-6).

Interferon gamma inducing factor (IGIF, interleukin-18, IL-18) is a recently described cytokine (25) that shares structural features with the interleukin-1 (IL-1) family of proteins (26). Activation of IGIF is mediated by interleukin-1 beta converting enzyme (ICE) (27, 28). Like IL-12, IGIF is a potent inducer of the production of IFN-γ by Th1 and natural killer (NK) cells, and acts on Th1 cells together with IL-12 in a synergistic manner (25, 29-32).

Experimental autoimmune encephalomyelitis (EAE) is a T cell mediated autoimmune disease of the central nervous system (CNS) which, for many years and for a variety of experimental protocols, serves as a model for the human disease, multiple sclerosis (MS), a chronic degenerative disease marked by patchy destruction of the myelin that surrounds and insulates nerve fibers and mild to severe neural and muscular impairments, since in both diseases circulating leukocytes penetrate the blood brain barrier and damage myelin resulting in impaired nerve conduction and paralysis (33, 34). Antigen specific T cells are thought to play a pivotal role in the manifestation of both diseases (35-37).

The role of Th1 cells in the manifestation of EAE has been widely studied. Th1 but not Th2 cells transfer the disease to normal naive recipients (18). Shifting the Th1/Th2 balance towards Th2 cells by *in vivo* administration of IL-4 (12), by antibodies to B7-1 (14), by soluble peptide therapy (38), or by administration of neutralizing antibodies to IL-12 (15) markedly suppressed EAE.

It has recently been shown that IGIF is a more potent inducer of IFN-γ producing Th1 cells than is IL-12 and thus plays an important role in Th1 responses (25). However, the possible role of anti-IGIF immunotherapy in regulation of T cell mediated autoimmunity has never been evaluated.

While reducing the present invention to practice it has been shown, for the first time, that neutralizing antibodies to IGIF ameliorate EAE by shifting the Th1/Th2 balance towards antigen specific Th2 cells.

SUMMARY OF THE INVENTION

The present invention disclosed the use of anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis. This use can be effected in a variety of ways as further described and exemplified hereinbelow.

According to one aspect of the present invention there is provided an antibody comprising an immunoglobulin capable of binding interferon gamma inducing factor.

According to another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an antibody being capable of binding an interferon gamma inducing factor.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding the interferon gamma inducing factor *in vivo*.

According to an additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to the animal cells being capable of producing and secreting an antibody

capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to yet additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to the animal an antibody capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to still additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to the animal an antigen including an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding *in vivo* an interferon gamma inducing factor.

According to further features in preferred embodiments of the invention described below, the antibody is polyclonal.

According to still further features in the described preferred embodiments the antibody is monoclonal.

According to still further features in the described preferred embodiments the antibody is a neutralizing antibodies to the interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to still further features in the described preferred embodiments the antibody is humanized.

According to another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to the animal a therapeutic composition including a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to yet another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the steps of (a) removing cells of the animal; (b) genetically modifying the cells *in vitro* with a recombinant construct including an isolated nucleic acid sequence encoding an interferon gamma inducing factor or an immunogenic portion thereof; and (c) reintroducing the genetically modified cells to the animal.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity

against multiple sclerosis, comprising a pharmaceutically acceptable carrier and a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to further features in preferred embodiments of the invention described below, the nucleic acid sequence being operatively linked to one or more transcription control sequences.

According to still further features in the described preferred embodiments the transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

According to still further features in the described preferred embodiments the recombinant construct is an eukaryotic expression vector.

According to still further features in the described preferred embodiments the recombinant construct is selected from the group consisting of pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES and their derivatives.

According to still further features in the described preferred embodiments the therapeutic composition is administered to the animal parenterally.

According to still further features in the described preferred embodiments the animal is a human being.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier comprises a delivery vehicle that delivers the nucleic acid sequences to the animal.

According to still further features in the described preferred embodiments the delivery vehicle is selected from the group consisting of liposomes, micelles, and cells.

According to still further features in the described preferred embodiments the recombinant construct is an eukaryotic expression vector.

According to still further features in the described preferred embodiments the composition is suitable for parenteral administration to a human.

The present invention successfully addresses the shortcomings of the presently known configurations by providing new horizons to the treatment of the devastating autoimmune disease multiple sclerosis.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIGs. 1A-F demonstrate IGIF mRNA in the inflamed EAE brain. Figures 1A, C, G and E - Rats were injected with 10^7 cells form L68-86 immunized rats to allow for the development of transferred EAE. Before adoptive transfer of disease (day 0), and at various time points: before the onset of disease (day 3), at the day of onset (day 5), the peak (day 7), following recovery (day 10), and 10 days after recovery (day 20) mid-brain and brain stem samples from six different rats at each time point were examined. mRNA was isolated from each sample and subjected to RT-PCR analysis using specific oligonucleotide primers constructed for IGIF (Figure 1C) and for IFN-γ (Figure 1E). Each amplification was calibrated to β-actin (Figure 1G) and verified by Southern Blot analysis. Southern bolt images were objectively assessed using an FujiFilm Thermal System. Figures 1B, D, F and H - Rats were immunized with p68-86/CFA and developed active EAE. Before the induction of disease (day 0), and at various time points: before the onset of disease (day 8), at the peak (day 13), and following recovery (day 21) mid-brain and brain stem samples from six different rats at each time point were examined for mRNA transcription for IGIF (Figure 1D) and IFN-γ (Figure 1F) and calibrated to β-actin (Figure 1H), as described above.

FIGs. 2A-D demonstrate that neutralizing antibodies to recombinant rat IGIF block IFN-γ production in cultured T cells. Spleen cells from naive (Figures 2A and 2B) or from p68-86/CFA primed (day 9) Lewis rats (Figures 2C and 2D) were cultured *in vitro* with either Con A (Figures 2A and 2B), or with 100 μM of MBP p68-86 (Figures 2C and 2D) with or without the addition of 100 ng/ml of rabbit anti-rat IGIF (IgG) neutralizing antibodies (Figures 2A and 2C) or with IgG from non-immunized rabbits (data not shown), with or without the addition of 400 ng/ml recombinant rat IGIF (Figures 2B and 2D). After 72 hours of stimulation IFN-γ levels were

determined in the culture supernatants by an ELISA assay. Results are the mean ± S.E. of triplicate cultures.

FIGs. 3A-B demonstrate that neutralizing antibodies to recombinant rat IGIF block the development of both active and transferred EAE. Figure 3A - Lewis rats were immunized with p68-86/CFA to induce active EAE and then separated into three groups of six rats each. Eight, ten and eleven days after induction of disease, these groups were injected IV with rabbit anti-rat IGIF (IgG fraction 100μg/rat), with IgG fraction purified from non-immunized rabbits (control IgG), or with PBS. The rats were then monitored daily for clinical signs of EAE by an observer blind to the treatment protocol. Results are presented as mean clinical score ± S.E. Figure 3B - Transferred EAE was induced as described above (Figure 1). Recipients were then separated into three groups of six rats each. Three, five and seven days after induction of disease these groups were injected as described above (Figure 3A) and monitored daily for clinical signs of EAE by an observer blind to the treatment protocol. Results are presented as mean clinical score ± S.E.

FIGs. 4A-D demonstrate alteration in IFN-γ and IL-4 production in EAE rats injected with anti-IGIF neutralizing antibodies. Lewis rats were immunized with p68-86/CFA to induce active EAE and separated into three groups. Five and seven days after disease induction these groups were injected IV with either rabbit anti-rat IGIF (IgG fraction 100 μg/rat), with purified IgG from non-immunized rabbits, or with PBS. Before the onset of disease (day 9) splenic T cells from three rats in each group were cultured with 100 μM MBP p68-86 for 72 hours in stimulation medium that was not (Figures 4A and 4B) or was (Figures 4C and 4D) supplemented with recombinant rat IL-4 (5 ng/ml). After 72 hours of stimulation, IFN-γ levels were determined in culture supernatants by an ELISA assay. Results are of triplicate cultures expressed as mean ± S.E.

FIG. 5 demonstrates alteration in TNF-α production in EAE rats injected with anti-IGIF neutralizing antibodies. Levels of TNF-α were determined in supernatants obtained in experiment described in Figures 4A and 4B, by an ELISA assay. Results are of triplicate cultures expressed as mean ± S.E.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of interferon gamma inducing factor (IGIF) based vaccines which can be used in the treatment of multiple sclerosis. Specifically, the present invention can be used to confer protective immunity against multiple sclerosis.

The principles and operation of the interferon gamma inducing factor (IGIF) based vaccines according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

In autoimmune conditions, T cells reactive to self-antigens escape elimination in the thymus, and are activated in the periphery where they can provoke damage to specific cells and organs. Perturbation of the balance between self-reactive T cells with different cytokine profiles may serve as an effective way of restraining the harmful effect of autoimmune T cells (12, 14-16, 20-22, 38, 41-43).

Cytokines present at the initiation of $CD4^+$ T cell responses determine whether a Th1 or a Th2 response will predominate (1-6). Thus, administration of IL-4 or of antibodies to IL-12 preferentially favors Th2 selection *in vivo* and thus serves as a powerful way to inhibit two different T cell mediated autoimmune diseases: EAE and IDDM (12, 15, 22)

It is well known that high levels of IFN-γ positively select for TNF-α secreting Th1 cells (6). A previous study showed that administration of anti-IL-12 neutralizing antibodies blocks EAE while inducing a marked reduction of both IFN-γ and TNF-α production (15). IFN-γ and TNF-α together then exhibit a synergistic effect on enhancing expression of adhesion molecules on endothelial cells (44), and on eliciting the inflammatory process, which can be reversed by either anti-adhesion molecule immunotherapy (45, 46), or by blocking TNF-α (44, 47-50). It was previously demonstrated that EAE resistance acquired by soluble antigen therapy can be reversed by anti-IL-4 neutralizing antibodies (38).

This further demonstrated the pivotal role of the Th1/Th2 balance in regulation of T cell mediated autoimmunity (38).

IGIF is a recently described cytokine (25) that shares structural features with the interleukin-1 (IL-1) family of proteins (26). Activation of IGIF is mediated by interleukin-1 beta converting enzyme (ICE) (27, 28). Like IL-12, IGIF is a potent inducer of IFN-γ from Th1 and NK cells, and acts on Th1 cells together with IL-12 in a synergistic manner (25, 29-32). IGIF actually has more potent IFN-γ inducing capabilities than IL-12 and apparently utilizes a distinct signal transduction pathway for its elicitation (25, 31, 32, 51). Little is known about the role of IGIF in T cell mediated autoimmune disease. A recent study used RT PCR to demonstrate that the active stage of autoimmune diabetes in NOD mice is associated with the expression of IGIF (52).

As further detailed in the Examples section below, while conceiving and reducing the present invention to practice, specific oligonucleotide primers were used to identify and isolate interferon gamma inducing factor (IGIF) from the brain of rats with developing experimental autoimmune encephalomyelitis (EAE), a T cell mediated autoimmune disease of the central nervous system (CNS) that serves as a model for multiple sclerosis (MS).

IGIF was highly transcribed in the brain at the onset and during the course of active EAE. PCR products encoding rat IGIF were used to generate the recombinant protein which was used to induce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of interferon gamma (IFN-γ) by primed T cells proliferating in response to their target myelin basic protein (MBP) epitope and by Con A activated T cells from naive donors.

When administered to rats during the development of either active or transferred EAE, these antibodies significantly blocked the development of disease.

Splenic T cells form protected rats were cultured with the encephalitogenic MBP epitope and evaluated for production of IL-4 and IFN-γ. These cells, which proliferated, exhibited a profound increase in IL-4 production, accompanied by a significant decrease in IFN-γ and TNF-α production.

An elevated expression of IGIF at the time when the secondary influx of autoimmune cells is apparent at the site of inflammation in the EAE brain (38, 39, 46, 53) is demonstrated herein for the first time. So are

neutralizing antibodies, which were generated against IGIF cloned from this site of inflamination, to block the disease by altering the *in vivo* Th1/Th2 balance in favor of Th2 selection. This alteration included a marked reduction in the production of IFN-γ, and, most importantly, TNF-α, a proinflammatory cytokine that plays a critical role in T cell mediated autoimmunity (44, 47-50).

An interesting observation is that both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN-γ production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope.

The direct role of IFN-γ in EAE is enigmatic. Grewal et al. have used a CD40L-deficient mice that carry a transgenic T cell receptor specific for MBP to demonstrate that EAE induction is IFN-γ dependent (54). On the other hand not only were mice lacking IFN-γ susceptible to induction of active EAE (55) but also antibodies to IFN-γ were found capable of enhancing this disease (56, 57). A recent study has demonstrated that IL-12 is directly involved in the generation of autoreactive Th1-cells that induce EAE, both in the presence and the absence of IFN-γ (58). However, it could well be that alteration the Th1/Th2 balance towards IL-4 secreting Th2 cells confers EAE resistance not because it leads to a reduced production of IFN-γ, but rather because it results in a reduced production of TNF-α accompanied by a marked increase in IL-4 production.

It has recently been suggested that IGIF primarily effects IFN-γ production by Th1 but not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease. Hence, as shown herein, *in vivo* administration of anti IGIF neutralizing antibodies notably shift the Th1/Th2 balance in antigen specific proliferating T cells towards Th2 response.

Thus, the present invention teaches the use of an anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis. This use can be effected in a variety of ways and applications, some of which are further described and exemplified hereinbelow.

According to one aspect of the present invention there is provided an antibody which comprises an immunoglobulin capable of binding interferon gamma inducing factor (IGIF, IL-18).

As used herein in the specification and in the claims section below, the terms "antibody" and "immunoglobulin", which are interchangeably used, refer to any of several classes of structurally related proteins that function as part of the immune response of an animal, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins. These terms further relate to chimeric immunoglobulins which are the expression products of fused genes derived from different species. These terms further relate to immunologically active derivatives of the above proteins, including, but not limited to, an F(ab')2 fragment, an Fab fragment, an Fv fragment, a heavy chain, a light chain, an unassociated mixture of a heavy chain and a light chain, a heterodimer consisting of a heavy chain and a light chain, a catalytic domain of a heavy chain, a catalytic domain of a light chain, a variable fragment of a light chain, a variable fragment of a heavy chain, and a single chain variant of the antibody. Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains. The immunoglobulin or antibody according to the present invention could also be a "humanized" antibody, in which, for example animal (say murine) variable regions are fused to human constant regions, or in which murine complementarity-determining regions are grafted onto a human antibody structure (Wilder, R.B. et al., J. Clin. Oncol., 14:1383-1400, 1996). Unlike, for example, animal derived antibodies, "humanized" antibodies often do not undergo an undesirable reaction with the immune system of the subject. The terms "sFv" and "single chain antigen binding protein" refer to a type of a fragment of an immunoglobulin, an example of which is sFv CC49 (Larson, S.M. et al., Cancer, 80:2458-68, 1997). As used herein, the term "humanized antibodies" also reads on antibodies produced by non-human cells or organisms genetically modified to include nucleic acid sequences encoding a functional portion of the human immune system, wherein the resulting antibodies are substantially identical to human antibodies in that they are encoded by human derived genes. However, the term "antibody", as used herein, further relates to soluble portions of receptors capable of specifically binding their respective protein ligands, which, in that respect, function like immunoglobulins.

As used herein and in the claims, the term "animal" refers to any organism with an immune system.

According to yet another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis. The composition comprises a pharmaceutically acceptable carrier and an antibody being capable of binding an interferon gamma inducing factor.

Alternatively, the composition according to the present invention comprises a pharmaceutically acceptable carrier and an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding the interferon gamma inducing factor *in vivo*.

As used herein the phrase "immunogenic portion" refers to an immunogenic proteinaceous compound, which may include, among optional additional components, a plurality of amino acid residues. The term "amino acid" is understood to include the 20 naturally occurring amino acid residues; those amino acid residues often modified post-translationally *in vivo*, including for example hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acid residues including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acid residues. The amino acid residues according to the present invention form a peptide. The latter is understood to include native peptides, including degradation products or synthetically synthesized peptides, and further to peptidomimetics, such as peptoids and semipeptoids, which are peptide analogs, which may have, for example, modifications rendering the peptides more stable or less immunogenic while contacting body fluids. Such modifications include, but are not limited to, cyclization, N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH_2-NH, CH_2-S, CH_2-S=O, O=C-NH, CH_2-O, CH_2-CH_2, S=C-NH, CH=CH or CF=CH, backbone modification and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein.

According to yet additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity

against multiple sclerosis. The method is effected by administering, to the animal, an antibody capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to still additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method is effected administering, to the animal, an antigen including an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding *in vivo* an interferon gamma inducing factor.

As used herein the term "antigen" refers to an immunogen including at least one immunogenic epitope, which is represented in the equivalent native peptide in a continuous or discontinuous fashion.

According to a preferred embodiment of the present invention, the antibody is polyclonal. Preparation of polyclonal antibodies is known in the art and further described in the Examples section hereinunder.

According to another preferred embodiment of the present invention, the antibody is monoclonal. Methods of producing and identifying monoclonal antibodies are well known in the art.

Monoclonal antibodies may be obtained by processes comprising the generation of a plurality of monoclonal antibodies to an antigen and screening the plurality of antibodies so generated to identify a monoclonal antibody that binds and/or neutralizes the peptide of interest, interferon gamma inducing factor in the present case. Monoclonal antibodies may be generated either *in vitro* or *in vivo*. In a related process, an animal is immunized with an antigen thereby generating antibody producing lymphocytes in said animal, antibody producing lymphocytes are removed from the animal, said lymphocytes are fused with myeloma cells to produce a plurality of immortalized hybridoma cells each of which produces monoclonal antibodies, the plurality of monoclonal antibodies is screened to identify a monoclonal antibody that binds the peptide, and the hybridoma producing the antibody is cloned and propagated. Animals are typically immunized with a mixture comprising a solution of the immunogen in a physiologically acceptable vehicle, and any suitable adjuvant, which achieves an enhanced immune response to the immunogen. By way of example, the primary immunization conveniently may be accomplished with a mixture of a solution of the immunogen and Freund's complete adjuvant, said mixture being prepared in the form of a water in oil emulsion. Typically the immunization may be administered to the animals

intramuscularly, intradermally, subcutaneously, intraperitoneally, into the footpads, or by any appropriate route of administration. The immunization schedule of the immunogen may be adapted as required, but customarily involves several subsequent or secondary immunizations using a milder adjuvant such as Freund's incomplete adjuvant. Antibody titers and specificity of binding to the hapten can be determined during the immunization schedule by any convenient method including by way of example radioimmunoassay, or enzyme linked immunoassay. Antibody activity assays can performed in vitro as further exemplified in the Examples section that follows. When suitable antibody titers are achieved, antibody producing lymphocytes from the immunized animals are obtained, and these are cultured, selected and cloned, as is known in the art. Typically, lymphocytes may be obtained in large numbers from the spleens of immunized animals, but they may also be retrieved from the circulation, the lymph nodes or other lymphoid organs. Lymphocytes are then fused with any suitable myeloma cell line, to yield hybridomas, as is well known in the art. Alternatively, lymphocytes may also be stimulated to grow in culture, and may be immortalized by methods known in the art including the exposure of these lymphocytes to a virus, a chemical or a nucleic acid such as an oncogene, according to established protocols. After fusion, the hybridomas are cultured under suitable culture conditions, for example in multiwell plates, and the culture supernatants are screened to identify cultures containing antibodies that recognize the hapten of choice. Hybridomas that secrete antibodies that recognize the hapten of choice are cloned by limiting dilution and expanded, under appropriate culture conditions. Monoclonal antibodies are purified and characterized in terms of immunoglobulin type binding affinity and in vivo or in vitro neutralizing activity.

The antibody according to the present invention, be it a poly- or monoclonal antibody, is a neutralizing antibody to interferon gamma inducing factor in affecting cells to produce interferon gamma, that is to say that the antibody interferes with the functionality of interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to an additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method according to the present invention is effected by administering, to the animal, cells capable of producing and secreting an antibody capable of *in vivo* neutralizing an interferon gamma

inducing factor in affecting cells to produce interferon gamma. To this end, cloned cDNAs encoding antibodies or fragments thereof are used to genetically modify receptive cells, to thereby render the cells antibody producing cells.

Cloning of cDNAs encoding antibodies or fragments thereof may be accomplished by several approaches known in the art. In the preferred approach, mRNA from clonal hybridoma cell lines which produce antibodies is employed as starting material. The cells are harvested and mRNA is extracted by standard methods known in the art. The cDNA is prepared by reverse transcription of the mRNA by standard methods known in the art. The cDNA for each chain of the immunoglobulin is cloned separately, and may be amplified by polymerase chain reaction using appropriate primers. The cDNA is then ligated into appropriate vectors by standard methods. The cDNA may be cloned into expression vectors and expressed separately in any convenient expression system, so that the properties of the expressed single chains of the antibodies may be determined. Alternatively, the individual chains may be expressed in the same cells which are then screened for the production of recombinant active antibodies. The method of using the invention will be modified in accordance with the system that is selected according to the current principles that are known in the art of recombinant protein production. According to the present invention the genetic information for the production of the antibody of interest is introduced into the cells by an appropriate vector as is known in the art or by any other acceptable means. The present invention provides information that will enable the skilled artisan to prepare constructs of genetic material comprising an open reading frame that encodes at least one chain of a novel antibody capable of binding IGIF. It will be appreciated that in certain embodiments it will suffice to produce an active fragment of the catalytic activity, for instance a Fab fragment of the intact antibody or even an Fv fragment thereof. In addition to the nucleic acid encoding the protein or polypeptide of choice, the constructs of the invention may comprise the following elements: a selectable marker, an origin of replication, a transcriptional promoter, a translation start site, a signal sequence for secretion of the product.

U.S. Pat. application No. 09/123,485, filed July 28, 1998, which is incorporated by reference as if fully set forth herein, teaches the effectiveness of DNA vaccines in inducing protective immunity against multiple sclerosis. In that application, DNA sequences encoding for a

variety of chemokines and for the cytokine tumor necrosis factor alpha, were shown to elicit protective immunity against both induced and transferred EAE, while the prior art teaches protective immunity against both induced and transferred EAE via passive vaccination (administration of antibodies).

Similarly, DNA vaccination in various forms, some of which are further detailed hereinunder, can be used to confer protective immunity against multiple sclerosis.

Thus, according to another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method is effected by administering to the animal a therapeutic composition including a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to yet another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method is effected by implementing the following method steps, in which, in a first step, cells are removed from the animal. In a second step, the cells are genetically modified *in vitro* with a recombinant construct including an isolated nucleic acid sequence encoding an interferon gamma inducing factor or an immunogenic portion thereof. Finally, the genetically modified cells are reintroduced to the animal.

As used herein in the specification and in the claims section below, the term "genetically modified" refers to a process of inserting nucleic acids into cells. The insertion may, for example, be effected by transformation, viral infection, injection, transfection, gene bombardment, electroporation or any other means effective in introducing nucleic acids into cells. Following the modification the nucleic acid is either integrated in all or part, to the cell's genome (DNA), or remains external to the cell's genome, thereby providing stably modified or transiently modified cells. The cells according to this method of the invention may be of any kind. Especially suitable cells are those readily removable, genetically modifiable, and reintroduceable cells, such as, but not limited to, cells of the various blood lineages, derived either from whole blood or from bone marrow, fibroblast cells, etc. The genetically modified cells are preferably reintroduced to the animal parenterally.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis. The composition comprises a pharmaceutically acceptable carrier and a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to a preferred embodiment of the present invention, the nucleic acid sequence is operatively linked to one or more transcription control sequences, such as, but not limited to, RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and/or β-actin control sequences. Preferably, the recombinant construct is an eukaryotic expression vector, such as, but not limited to, pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES, and their derivatives.

According to a preferred embodiment of the present invention, the therapeutic composition is administered to the animal parenterally. According to another preferred embodiment of the present invention, the animal is a human being.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles. Preferably, the pharmaceutically acceptable carrier comprises a delivery vehicle that delivers the nucleic acid sequences to the animal. The delivery vehicle can be effected by liposomes, micelles or cells.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Materials and Experimental Methods

Rats: Female Lewis rats, six weeks old, were purchased from Harlan (Israel) and maintained under SPF conditions in an animal facility.

Peptide antigens: Myelin Basic Protein (MBP) p68-86, Y G S L P Q K S Q R S Q D E N P V (SEQ ID NO:1), was synthesized on a MilliGen 9050 peptide synthesizer by standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by high performance liquid chromatography. Structure was confirmed by amino acid analysis and mass spectroscopy. Only peptides that were greater than 95 % pure were used in our study.

Immunizations and induction of active disease: Rats were immunized subcutaneously in the hind foot pads with 0.1 ml of MBP epitope 68-86 (p68-86) dissolved in PBS (1.5 mg/ml) and emulsified with an equal volume of CFA (incomplete Freund's adjuvant supplemented with 4 mg/ml heat-killed *Mycobacterium tuberculosis* H37Ra in oil (Difco laboratories, Inc., Detroit, MI). Rats were then monitored for clinical signs daily by an observer blind to the treatment protocol. EAE was scored as follows: 0, clinically normal; 1, flaccid tail; 2, hind limb paralysis; 3, front and hind limb paralysis.

Induction of transferred EAE: EAE was induced by immunizing Lewis rats (intraperitoneally) with 10^8 activated spleen cells from EAE donors obtained as follows: Nine days after induction of active EAE, splenic cells were cultured (12 x 10^6/ml) at 37 °C in humidified air containing 7.5 % CO_2 for two days in stimulation medium that includes Dulbecco's modified Eagle's medium (Gibco) supplemented with 2-mercaptoethanol (5 x 10^{-5} M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 µg/ml), streptomycin (100 µg/ml), 1 % syngeneic serum and 20-30 µg/ml of the immunizing epitope. Then, cells were separated on a Ficoll gradient (Sigma), resuspended in PBS and injected to naive recipients.

Antigen-specific T cell proliferation assays: Lewis rats were immunized with MBP p68-86/CFA as described above. Nine to ten days later spleen cells were suspended in stimulation medium and cultured in U-

shape 96-well microculture plates (2 x 10^5 cells/well) for 72 hours, at 37 °C in humidified air containing 7.5 % CO_2. Each well was pulsed with 2 µCi of [^{3}H]-Thymidine (specific activity 10 Ci/mmol) for the final six hours. The cultures were then harvested on fiberglass filters and the proliferative response expressed as CPM ± S.E. or as stimulation index (SI) (mean CPM of test cultures divided by mean CPM of control cultures).

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis: RT-PCR analysis, verified by Southern blotting, was utilized on brain samples according to the protocol described elsewhere with some modifications (39). Rats were euthanized by CO_2 narcosis. Brain samples containing mainly the midbrain and brain stem were obtained after perfusion of the rat with 160-180 ml of ice-cold phosphate buffered saline (PBS) injected into the left ventricle following an incision in the right atrium. Each sample was homogenized. Total RNA was extracted using the Tri-Zol procedure (Gibco BRL) according to the manufacturer's protocol. mRNA was then isolated using a mRNA isolation kit (#1741985, Boheringer Mannheim, Germany), and reverse transcribed into first strand cDNA as described in detail elsewhere (39). First strand cDNA was then subjected to 35 cycles of PCR amplification using specific oligonucleotide primers to rat IGIF and IFN-γ which were designed based on the published sequence of each cytokine (NCBI accession number for rat IGIF - U77777; and for rat IFN-γ - M29315) as follows (Table 1):

TABLE 1

Name/Function	Sequence	SEQ ID NO:
Rat IGIF sense	5'-ATGGCTGCCATGTCAGAAGAAG-3'	2
Rat IGIF antisense	5'-CTAACTTTGATGTAAGTTAGTAAGA-3'	3
Rat IFN-γ sense	5'-TACTGCCAAGGCACACTCATTGAA-3'	4
Rat IFN-γ antisense	5'-CGCTTCCTTAGGCTAGATTCTGG-3'	5
Rat β-actin sense	5'-CATCGTGGGCCGCTCTAGGCA-3' *	6
Rat β-actin antisense	5'-CCGGCCAGCCAAGTCCAGACG-3' *	7

Sequence according to Reference 39.

Experimental conditions were calibrated so RT-PCR amplifications fall on the linear part of the titration curve. The cycle profile was: denaturation at 95 °C for 60 seconds, annealing at 55 °C for 60 seconds, and

elongation at 72 °C for 60 seconds. Amplified products were subjected to electrophoresis, transferred to a nylon membranes (MagnaGraph nylon transfer membrane, msi, Westborough, MA), fixed with ultraviolet light (120 mjoules) and hybridized with 10^6 cpm/ml of a ^{32}P labeled DNA fragments encoding the full length PCR product of IGIF and of β-actin (random priming: Amersham, Arlington Heights, IL). PCR products were used as probes only after each PCR product was cloned and its sequence was verified as described below. Southern bolt images were objectively assessed using an FujiFilm Thermal System FTI-500 (FujiFilm, Japan).

Cloning and sequencing of PCR products: Each of the amplified PCR products described above was cloned into a pUC57/T vector (T-cloning Kit #K1212, MBI Fermentas, Lithuania) and transformed to *E. coli* according to the manufacturer's protocol. Each clone was then sequenced (Sequenase version 2, USB, Cleveland, Ohio) according to the manufacturer's protocol.

Production and purification of recombinant proteins. After sequence verification, PCR products were recloned into a PQE expression vector (PQE-30, PQE-31 or PQE-32 according the correct open reading frame) and expressed in E. Coli (Qaigen, Hilden, GmbH) and then purified by an NI-NTA-super flow affinity purification of 6 x His proteins (Qaigen). Each recombinant protein sequence has been verified (N -terminus).

Production and purification of Rabbit anti-rat IGIF IgG: Rabbit anti-rat IGIF antibodies were generated as described (40) and IgG fraction was purified using a HiTrap protein G kit (Pharmacia, Piscataway, NJ, Kit #17-040-01). Antibody titer was determined by a direct ELISA assay: ELISA plates (Nunc, Denmark) were coated with recombinant rat IGIF(50 ng/well). Rabbit anti-rat IGIF (IgG fraction) was added in serial dilutions from 2^8 to 2^{30}. Goat anti-rabbit IgG alkaline phosphatase conjugated antibodies (Sigma) were used as a labeled antibody. p-Nitrophenyl Phosphate(p-NPP) (Sigma) was used as a soluble alkaline phosphatase substrate. Results of triplicates were calculated as log 2 antibody titer ± SE. The purified anti-rat IGIF IgG titer was 18±0.4.

Cytokine determination: Spleen cells from EAE donors were stimulated *in vitro* (10^7 cells/ml) in 24 well plates (Nunc) with 100 μM p68-86. Spleen cells from naive donors were cultured (10^7 cells/ml, 24 well plates) with 2 μg/ml Con A (Sigma). After 72 hours of stimulation, supernatants were assayed by semi-ELISA kits, that include antibody pairs and recombinant rat cytokines, as follows: IFN-γ, rabbit anti-rat IFN-γ

polyclonal antibody (CY-048, Innogenetics, Belgium) as a capture antibody, biotinylated mouse anti-rat monoclonal antibody (CY-106 clone BD-1, Innogenetics) as a detection antibody, and Alkaline phosphatase-Streptavidin (cat No. 43-4322, Zymed, SF, CA) with rat recombinant IFN-γ as a standard (Cat No 3281SA, Gibco BRL); TNF-α, commercial semi-ELISA kit for the detection of rat TNF-α, (Cat No 80-3807-00, Genzyme, Cambridge, MA); IL-4, mouse anti-rat IL-4 monoclonal antibody (24050D OX-81, PharMingen, San Diego, CA) as a capture antibody, and rabbit anti-rat IL-4 biotin-conjugated polyclonal antibody (2411-2D, PharMingen) as second antibody. Recombinant rat IL-4 purchased from R&D (504-RL) was used as a standard.

Statistical analysis: Significance of differences was examined using Student's t-test. Mann-Whitney sum of ranks test was used to evaluate significance of differences in mean of maximal clinical score (Figure 3). Value of $p < 0.05$ was considered significant.

Experimental Results

IGIF mRNA is transcribed in the inflamed EAE brain: Midbrain-brain stem samples were obtained from rats with developing transferred EAE (Figure 1A) before adoptive transfer of disease (day 0), and at various time points: before the onset of disease (day 3), at the day of onset (day 5), the peak (day 7), following recovery (day 10), and 10 days after recovery (day 20). For each time point, samples from six different brains were subjected to RT-PCR analysis using specific oligonucleotide primers which constructed to IGIF and IFN-γ . Each amplification was calibrated to β-actin and verified by Southern blot analysis. This enabled semi-quantitative analysis of the dynamics of mRNA transcription of IGIF and IFN-γ at the site of inflammation. Figures 1C and 1E show representative results from each time point of the experiment. A substantial increase in the transcription of both IGIF and IFN-γ mRNA in EAE brains was observed at the peak of disease (day 7). The augmented transcription of IFN-γ mRNA reverted to background levels during recovery. Unexpectedly, a notable transcription of IGIF mRNA could be observed even ten days after recovery (Figure 1C).

Rats with developing active disease manifested similar mRNA transcription characteristics as those with developing transferred disease. That is, a substantial increase in the transcription of both IGIF and IFN-γ mRNA in EAE brains was observed at the peak of disease (day 13). The

augmented transcription of IFN-γ, but not of IGIF mRNA, regressed to background level during recovery (Figures 1D and 1F). A substantial increase in the level of a IGIF transcription at the site of inflammation in the CNS during the course of disease may suggest involvement in its regulation. To evaluate this point, the role of IGIF in regulation of EAE was investigated.

Recombinant rat IGIF and its neutralizing antibodies affect IFN-γ production by activated T cells from naive donors more significantly than by antigen specific primed T cells: PCR products encoding rat IGIF were used to generate the recombinant protein which was used to produce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of IFN-γ in primed T cells proliferating in response to their specific myelin basic protein (MBP) epitope (Figure 2C 3.2 ± 0.25 versus 1.8 ± 0.11 ng/ml with backgrounds of 0.2 ± 0.1 and 0.25 ± 0.1, $p < 0.01$) and entirely blocked IFN-γ production in Con A activated T cells from naive donors (Figure 2A, 5.1 ± 0.4 versus 0.4 2 ± 0.1 ng/ml with backgrounds of 0.4 ± 0.1 and 0.36, $p < 0.001$). Control IgG from normal rabbit serum did not exert a notable effect on IFN-γ production by either Con A activated naive spleen cells or MBP p68-86 primed spleen cells (data not shown). Recombinant rat IGIF elicited IFN-γ production in Con A activated splenic T cells from naive donors (Figure 2B, 15.8 ± 0.8 ng/ml versus 5.1 ± 0.3 with backgrounds of 0.3 ± 0.1 and 0.4 ± 0.15, $p < 0.001$) and significantly, though again less profoundly, the response of primed spleen T cells to their target MBP antigen (Figure 2D, 4.97 ± 0.15 ng/ml versus 3.2 ± 0.25, with backgrounds of 0.3 ± 0.15 and 0.25 ± 0.1, $p < 0.001$). Thus, both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN-γ production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope. It has recently been suggested that IGIF primarily affects IFN-γ production by Th1 not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease.

The *in vitro* addition of either anti-IGIF antibodies or of recombinant IGIF did not affect the antigen specific proliferative response developed in primed splenic T cells responding to MBP p68-86 (SI = 4.2 ± 0.3, 3.6 ± 0.4 and 3.9 ± 0.3 in control spleen T cells) versus cultured spleen cells supplemented with either anti-IGIF antibodies or recombinant IGIF.

Neutralizing antibodies to recombinant rat IGIF block the development of both active and transferred EAE: The role of anti-IGIF antibodies in the regulation of T cell mediated autoimmune diseases has never been explored before. Herein the competence of the anti-IGIF neutralizing antibodies to inhibit active (Figure 3A) and transferred (Figure 3B) EAE is evaluated. Lewis rats were immunized with p68-86/CFA to develop active EAE. Just before the onset of disease (days eight and ten) and at the onset of disease (day eleven) these rats were injected with either rabbit anti-rat IGIF (IgG fraction), IgG fraction purified from non-immunized rabbits (control IgG), or with PBS, and monitored for clinical signs of EAE. Control PBS treated rats and rats treated with control IgG all (6/6 rats in each group) developed severe EAE (Mean maximal clinical score 3.3 ± 0.43 and 2.66 ± 0.26, respectively). In contrast, rats treated with anti-IGIF antibodies developed a markedly reduced disease (Figure 3A, incidence 5/6, mean maximal clinical score 1.2 ± 0.2, $p < 0.01$).

The competence of anti-IGIF antibodies to inhibit transferred EAE (Figure 3B) was further evaluated. Three, five and seven days after adoptive transfer of disease rats were injected as described above and monitored for clinical signs of EAE. While control PBS treated rats and rats treated with control IgG have all (6/6 rats in each group) developed EAE (mean maximal clinical score 1±0 in each group) rats administered with anti-IGIF antibodies were highly protected (Figure 3B, incidence 1/6, mean maximal clinical score 0.2 ± 0.1, $p < 0.01$). Thus, immunotherapy with anti-IGIF serves as a powerful tool to block the development of actively induced or transferred EAE.

Alteration of IFN-γ and IL-4 production in EAE rats injected with anti-IGIF neutralizing antibodies suggests that perturbation of the Th2/Th1 balance contributes to disease blockade: The possible involvement of a Th2/Th1 switch in EAE inhibition by anti-IGIF immunotherapy has been evaluated (Figure 4). Lewis rats were immunized with p68-86/CFA to develop active EAE. Five and seven days later these rats were injected with either PBS, control rabbit IgG or rabbit anti-rat IGIF (IgG fraction). Two days after the last treatment, splenic T cells were cultured with MBP p68-86 in stimulation medium that was (Figures 4C and 4D) or was not supplemented with recombinant rat IL-4 (Figures 4A and 4B). In spleen cells cultured from MBP 68-86 primed donors, IFN-γ was produced only when the priming antigen was added to the culture (Figures 4A, 0.3 ± 0.1 ng/ml without addition of MBP 68-86 versus 13.5 ± 0.7 in

cells proliferating to p68-86). Addition of recombinant IL-4 led to a significant decrease in IFN-γ which was still dependent upon antigenic stimulation (Figures 4A and 4C 0.19 ± 0.08 ng/ml without addition of MBP 68-86 versus 2.37 ± 0.8 in cells proliferating to p68-86, a 12 fold increase). Spleen T cells from anti-IGIF treated rats produced markedly reduced levels of IFN-γ in response to antigenic stimulation in cultures that were or were not supplemented with IL-4 (Figure 4A, 4.7 ± 0.4 ng/ml in spleen cells from anti-IGIF treated rat versus 9.7 ± 0.8 in spleen cells from rats treated with normal rabbit IgG and 13.5 ± 0.7 in PBS treated rats, with backgrounds of 0.4, 0.8 and 0.7, $p < 0.001$, when comparing anti-IGIF treatment to each control group). IL-4 production, however, markedly increased in splenic T cells from anti-IGIF treated rats regardless of *in vitro* stimulation (Figure 4B, 62.3 ± 4.2 pg/ml in spleen cells from anti-IGIF treated rat versus 15.3 ± 0.4 in spleen cells from rats treated with normal rabbit IgG and 15.6 ± 0.6 in PBS treated rats , $p < 0.001$, when comparing anti-IGIF treatment to each control group) unless cultures were supplemented with IL-4 (Figure 4D, 1860 ± 120 pg/ml in spleen cells from anti-IGIF treated rat versus 570 ± 30 in spleen cells from rats treated with normal rabbit IgG and 450 ± 35 in PBS treated rats, with backgrounds of 85, 42 and 34, $p < 0.0001$, when comparing anti-IGIF treatment to each control group). Addition of IL-4 to cultured spleen T cells (Figure 4C-D) did not exhibit a notable effect on their antigen specific proliferative response (data not shown).

TNF-α production was then evaluated in spleen cells from the above groups. The above spleen cells from anti-IGIF treated rats produced markedly reduced levels of TNF-α in response to antigenic stimulation (Figure 5, 850 ± 45 pg/ml in spleen cells from anti-IGIF treated rats versus 1975 ± 80 in spleen cells from rats treated with normal rabbit IgG and 2100 ± 110 in PBS treated rats, with backgrounds of 230, 210 and 270, respectively, $p < 0.001$, when comparing anti-IGIF treatment to each control group). Thus perturbation of the Th1/Th2 balance in anti-IGIF treated rats is associated with a marked reduction in TNF-α production.

Finally, the proliferative response of each group of cultured spleen cells to p68-86 was evaluated in a proliferation assay. (SI = 4.2 ± 0.3, 3.6 ± 0.4 and 3.96 ± 0.5 in spleen cells from rats treated with either anti-IGIF, normal rabbit IgG or PBS respectively). Thus anti-IGIF immunotherapy alters Th2/Th1 balance without a notable affect on antigen specific proliferative responsiveness.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

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